For IFW

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L2 ANSWER 1 OF 9 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:18837 HCAPLUS
DOCUMENT NUMBER: 140:92683
TITLE: Preparation of amorpha-4,11-diene with transgenic microorganisms producing isopentenyl- and dimethylallyl pyrophosphates
INVENTOR(S): Keasling, Jay; Martin, Vincent; Pitera, Douglas; Withers, Sydnor T.; Newman, Jack
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 75 pp., Cont.-in-part of U.S. Ser. No. 6,909.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004005678	A1	20040108	US 2003-411066	20030409
US 2003148479	A1	20030807	US 2001-6909	20011206

PRIORITY APPLN. INFO.: US 2001-6909 A2 20011206
AB Methods for synthesizing amorpha-4,11-diene from isopentenyl pyrophosphate are provided. A first method comprises introducing into a host microorganism a plurality of heterologous nucleic acid sequences, each coding for a different enzyme in the mevalonate pathway for producing isopentenyl pyrophosphate. Amorpha-4,11-diene is then produced with the transgenic microorganism which is further transformed with an optimized **amorpha-4,11-diene synthase** gene. The amorpha-4,11-diene may be used in synthesis of the antimalarial drug artemisinin. Thus, amorpha-4,11-diene was prepd. from mevalonate supplied in the medium with Escherichia coli transformed with plasmid pBBRMDIS-2, contg. the yeast genes idi (for isopentenyl pyrophosphate isomerase) and ispA (for farnesyl pyrophosphate synthase) and the genes for mevalonate kinase, phosphomevalonate kinase, mevalonate pyrophosphate decarboxylase, and **amorpha-4,11-diene synthase**. The yield was 2 .mu.g amorpha-4,11-diene/mL.

L2 ANSWER 2 OF 9 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:609986 HCAPLUS
DOCUMENT NUMBER: 139:160786
TITLE: Biosynthesis of isopentenyl pyrophosphate using recombinant microbial metabolic pathways
INVENTOR(S): Keasling, Jay; Martin, Vincent; Pitera, Douglas; Kim, Seon-Won; Withers, Sydnor T.; Yoshikuni, Yasuo; Newman, Jack; Khlebnikov, Artem Valentinovich
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 40 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003148479	A1	20030807	US 2001-6909	20011206
US 2004005678	A1	20040108	US 2003-411066	20030409

PRIORITY APPLN. INFO.: US 2001-6909 A2 20011206
AB Methods for synthesizing isopentenyl pyrophosphate are provided. A first method comprises introducing into a host microorganism a plurality of heterologous nucleic acid sequences, each coding for a different enzyme in

the mevalonate pathway for producing isopentenyl pyrophosphate. A related method comprises introducing into a host microorganism an intermediate in the mevalonate pathway and at least one heterologous nucleic acid sequence, each sequence coding for an enzyme in the mevalonate pathway necessary for converting the intermediate into isopentenyl pyrophosphate. The invention also provides nucleic acid sequences, enzymes, expression vectors, and transformed host cells for carrying out the methods.

L2 ANSWER 3 OF 9 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2003324605 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12778056
TITLE: Engineering a mevalonate pathway in Escherichia coli for production of terpenoids.
AUTHOR: Martin Vincent J J; Pitera Douglas J; Withers Sydnor T; Newman Jack D; Keasling Jay D
CORPORATE SOURCE: Department of Chemical Engineering, 201 Gilman Hall, University of California, Berkeley, California 94720-1462, USA.
SOURCE: Nature biotechnology, (2003 Jul) 21 (7) 796-802. Journal code: 9604648. ISSN: 1087-0156.
PUB. COUNTRY: United States
DOCUMENT TYPE: (EVALUATION STUDIES) Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200404
ENTRY DATE: Entered STN: 20030713 Last Updated on STN: 20040407 Entered Medline: 20040406

AB Isoprenoids are the most numerous and structurally diverse family of natural products. Terpenoids, a class of isoprenoids often isolated from plants, are used as commercial flavor and fragrance compounds and antimalarial or anticancer drugs. Because plant tissue extractions typically yield low terpenoid concentrations, we sought an alternative method to produce high-value terpenoid compounds, such as the antimalarial drug artemisinin, in a microbial host. We engineered the expression of a synthetic **amorpha-4,11-diene synthase** gene and the mevalonate isoprenoid pathway from Saccharomyces cerevisiae in Escherichia coli. Concentrations of amorphadiene, the sesquiterpene olefin precursor to artemisinin, reached 24 microg caryophyllene equivalent/ml. Because isopentenyl and dimethylallyl pyrophosphates are the universal precursors to all isoprenoids, the strains developed in this study can serve as platform hosts for the production of any terpenoid compound for which a terpene synthase gene is available.

L2 ANSWER 4 OF 9 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:187822 HCAPLUS
TITLE: Cloning, E. coli expression and molecular analysis of a novel sesquiterpene synthase gene from Artemisia annua
AUTHOR(S): Liu, Yan; Ye, Hechun; Li, Guofeng
CORPORATE SOURCE: Key laboratory of Plant Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, Peop. Rep. China
SOURCE: Zhiwu Xuebao (2002), 44(12), 1450-1455 CODEN: CHWHAY; ISSN: 0577-7496
PUBLISHER: Kexue Chubanshe
DOCUMENT TYPE: Journal
LANGUAGE: English

AB 1 886 bp full-length sesquiterpene synthase (AaSES) cDNA was cloned from a high-yield Artemisia annua L. strain 001 by a rapid amplification of cDNA end (RACE) strategy. AaSES was 59% identical to Artemisia cyclase cDNA clone cASC125, 50% identical to epi-cedrol synthase from A. annua, 48%

identical to **amorpha-4,11-diene synthase** from A. annua, 39% identical to the 5-epi-aristolechene synthase from tobacco, 38% identical to vetispiradiene synthase from H. muticus, 41 % identical to the .delta.-cadinene synthase from cotton. The coding region of the cDNA was inserted into a procaryotic expression vector pET-30a and overexpressed in E. coli BL21 (DE3). The cyclase proteins extd. from bacterial culture were found largely in an insol. protein fraction. AaSES expressed in leaves, stems and flowers, not in roots as indicated by Northern blotting anal.

L2 ANSWER 5 OF 9 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2001197498 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11289612
TITLE: **Amorpha-4,11-diene synthase**: cloning and functional expression of a key enzyme in the biosynthetic pathway of the novel antimalarial drug artemisinin.
AUTHOR: Wallaart T E; Bouwmeester H J; Hille J; Poppinga L; Maijers N C
CORPORATE SOURCE: GenoClipp Biotechnology BV, Meditech Center, Groningen, The Netherlands.. mail@genoclipp.com
SOURCE: Planta, (2001 Feb) 212 (3) 460-5.
Journal code: 1250576. ISSN: 0032-0935.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AY006482
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010723
Last Updated on STN: 20010723
Entered Medline: 20010719

AB The sesquiterpenoid artemisinin, isolated these from the plant Artemisia annua L., and its semi-synthetic derivatives are a new and very effective group of antimalarial drugs. A branch point in the biosynthesis of this compound is the cyclisation of the ubiquitous precursor farnesyl diphosphate into the first specific precursor of artemisinin, namely amorpha-4,11-diene. Here we describe the isolation of a cDNA clone encoding **amorpha-4,11-diene synthase**. The deduced amino acid sequence exhibits the highest identity (50%) with a putative sesquiterpene cyclase of A. annua. When expressed in Escherichia coli, the recombinant enzyme catalyses the formation of amorpha-4,11-diene from farnesyl diphosphate. Introduction of the gene into tobacco (Nicotiana tabacum L.) resulted in the expression of an active enzyme and the accumulation of amorpha-4,11-diene ranging from 0.2 to 1.7 ng per g fresh weight.

L2 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:144616 HCAPLUS
DOCUMENT NUMBER: 132:204840
TITLE: Artemisia annua **amorpha-4, 11-diene synthase**, its cDNA, recombinant expression, and methods of amorpha-4,11-diene and artemisinin synthesis via transgenic plants
INVENTOR(S): Wallaart, Thorvald Eelco; Bouwmeester, Hendrik Jan
PATENT ASSIGNEE(S): Neth.
SOURCE: Eur. Pat. Appl., 41 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

EP 982404	A1	20000301	EP 1998-202854	19980827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
CA 2340925	AA	20000309	CA 1999-2340925	19990827
WO 2000012725	A2	20000309	WO 1999-EP6302	19990827
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9957423	A1	20000321	AU 1999-57423	19990827
AU 766764	B2	20031023		
EP 1108041	A2	20010620	EP 1999-944535	19990827
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
BR 9913196	A	20010925	BR 1999-13196	19990827
JP 2002523101	T2	20020730	JP 2000-567711	19990827
ZA 2001001455	A	20010828	ZA 2001-1455	20010221

PRIORITY APPLN. INFO.: EP 1998-202854 A 19980827
WO 1999-EP6302 W 19990827

AB **Amorpha-4,11-diene**
synthase from Artemisia annua L., its cDNA, recombinant expression, and methods of prepg. amorpha-4,11-diene and artemisinin from farnesyl pyrophosphate (FPP) using transgenic organism are provided. Amorpha-4,11-diene is a precursor of the new anti-malarial drug artemisinin produced by the plant Artemisia annua L. A cDNA encoding **amorpha-4,11-diene synthase** from A. annua has been isolated and sequenced, and the corresponding amino acid sequence has been detd. Recombinant **amorpha-4, 11-diene synthase** expressed in E. coli, transgenic tobacco, and transgenic A. annua catalyzed conversion of FPP into amorpha-4,11-diene. Further conversion of amorpha-4,11-diene into artemisinin was obsd. in transgenic A. annua. The invention may be useful in obtaining enhanced prodn. of stereochem. desirable artemisinin.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 7 OF 9 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2001128077 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11185551
TITLE: **Amorpha-4,11-diene**
synthase of Artemisia annua: cDNA isolation and bacterial expression of a terpene synthase involved in artemisinin biosynthesis.
AUTHOR: Chang Y J; Song S H; Park S H; Kim S U
CORPORATE SOURCE: School of Agricultural Biotechnology and the Research Center for New Biomaterials in Agriculture, Seoul National University, Suwon, Korea.
SOURCE: Archives of biochemistry and biophysics, (2000 Nov 15) 383 (2) 178-84.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ251751
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010301

AB Artemisia annua, an indigenous plant to Korea, contains an antimalarial sesquiterpene, artemisinin. The first committed step of artemisinin biosynthesis is the cyclization of farnesyl diphosphate by a sesquiterpene synthase to produce an amorphane-type ring system. The aims of this research were to molecularly clone and express **amorpha-4,11-diene synthase** for metabolic engineering. PCR amplification of genomic DNA with a pair of primers, designed from the conserved regions of sesquiterpene synthases of several plants, produced a 184-bp DNA fragment. This fragment was used in Northern blot analysis as a probe, showing approximately 2.2 kb of a single band. Its sequence information was used to produce 2106 bp of a full-length cDNA sequence including 1641 bp of open reading frame for 546 amino acids (kcs12) through a rapid amplification of cDNA ends (RACE). The deduced amino acid sequence displayed 36% identity with 5-epi-aristolochene synthase of Nicotiana tabacum. A soluble fraction of Escherichia coli harboring kcs12 catalyzed the cyclization of farnesyl diphosphate to produce a sesquiterpene, which was identified through GC-MS analysis as amorpha-4,11-diene.

L2 ANSWER 8 OF 9 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2000479808 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11032404
TITLE: Molecular cloning, expression, and characterization of **amorpha-4,11-diene synthase**, a key enzyme of artemisinin biosynthesis in Artemisia annua L.
AUTHOR: Mercke P; Bengtsson M; Bouwmeester H J; Posthumus M A; Brodelius P E
CORPORATE SOURCE: Department of Plant Biochemistry, Lund University, Sweden.
SOURCE: Archives of biochemistry and biophysics, (2000 Sep 15) 381 (2) 173-80.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF138959
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001031

AB In plants, sesquiterpenes of different structural types are biosynthesized from the isoprenoid intermediate farnesyl diphosphate. The initial reaction of the biosynthesis is catalyzed by sesquiterpene cyclases (synthases). In Artemisia annua L. (annual wormwood), a number of such sesquiterpene cyclases are active. We have isolated a cDNA clone encoding one of these, **amorpha-4,11-diene synthase**, a putative key enzyme of artemisinin biosynthesis. This clone contains a 1641-bp open reading frame coding for 546 amino acids (63.9 kDa), a 12-bp 5'-untranslated end, and a 427-bp 3'-untranslated sequence. The deduced amino acid sequence is 32 to 51% identical with the sequence of other known sesquiterpene cyclases from angiosperms. When expressed in Escherichia coli, the recombinant enzyme catalyzed the formation of both olefinic (97.5%) and oxygenated (2.5%) sesquiterpenes from farnesyl diphosphate. GC-MS analysis identified the olefins as (E)-beta-farnesene (0.8%), amorpha-4,11diene (91.2%), amorpha-4,7(11)-diene (3.7%), gamma-humulene (1.0%), beta-sesquiphellandrene (0.5%), and an unknown olefin (0.2%) and the oxygenated sesquiterpenes as amorpha-4-en-11-ol (0.2%) (tentatively), amorpha-4-en-7-ol (2.1%), and alpha-bisabolol (0.3%) (tentatively). Using geranyl diphosphate as substrate, **amorpha-4,11-diene synthase** did not produce any monoterpenes. The recombinant enzyme has a broad pH optimum between 7.5 and 9.0 and the Km values for farnesyl diphosphate, Mg2+, and Mn2+ are 0.9, 70, and 13 microM, respectively, at pH 7.5. A putative reaction mechanism for **amorpha-4,**

11-diene synthase is suggested.

L2 ANSWER 9 OF 9 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2000091820 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10626375
TITLE: **Amorpha-4,11-diene synthase** catalyses the first probable step in artemisinin biosynthesis.
AUTHOR: Bouwmeester H J; Wallaart T E; Janssen M H; van Loo B; Jansen B J; Posthumus M A; Schmidt C O; De Kraker J W; Konig W A; Franssen M C
CORPORATE SOURCE: Research Institute for Agrobiology and Soil Fertility (AB-DLO), Wageningen, Netherlands.. h.j.bouwmeester@ab.dlo.nl
SOURCE: Phytochemistry, (1999 Nov) 52 (5) 843-54. Journal code: 0151434. ISSN: 0031-9422.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000229
Last Updated on STN: 20000229
Entered Medline: 20000211

AB The endoperoxide sesquiterpene lactone artemisinin and its derivatives are a promising new group of drugs against malaria. Artemisinin is a constituent of the annual herb Artemisia annua L. So far only the later steps in artemisinin biosynthesis--from artemisinic acid--have been elucidated and the expected olefinic sesquiterpene intermediate has never been demonstrated. In pentane extracts of A. annua leaves we detected a sesquiterpene with the mass spectrum of amorpha-4,11-diene. Synthesis of amorpha-4,11-diene from artemisinic acid confirmed the identity. In addition we identified several sesquiterpene synthases of which one of the major activities catalysed the formation of amorpha-4,11-diene from farnesyl diphosphate. This enzyme was partially purified and shows the typical characteristics of sesquiterpene synthases, such as a broad pH optimum around 6.5-7.0, a molecular mass of 56 kDa, and a K(m) of 0.6 microM. The structure and configuration of amorpha-4,11-diene, its low content in A. annua and the high activity of **amorpha-4,11-diene synthase** all support that amorpha-4,11-diene is the likely olefinic sesquiterpene intermediate in the biosynthesis of artemisinin.

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=> s amorphadiene synthase
L3            4 AMORPHADIENE SYNTHASE

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4            4 DUP REM L3 (0 DUPLICATES REMOVED)

=> d l4 1-4 ibib ab
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L4 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:109756 HCAPLUS
DOCUMENT NUMBER: 134:338261
TITLE: Amorpha-4,11-diene synthase: cloning and functional expression of a key enzyme in the biosynthetic pathway of the novel antimalarial drug artemisinin
AUTHOR(S): Wallaart, T. Eelco; Bouwmeester, Harro J.; Hille, Jacques; Poppinga, Lucas; Maijers, Niels C. A.
CORPORATE SOURCE: GenoClipp biotechnology B.V., Meditech Center, Groningen, 9713 GX, Neth.
SOURCE: Planta (2001), 212(3), 460-465
CODEN: PLANAB; ISSN: 0032-0935

PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The sesquiterpenoid artemisinin, isolated from the plant Artemisia annua L., and its semi-synthetic derivs. are a new and very effective group of antimalarial drugs. A branch point in the biosynthesis of this compd. is the cyclization of the ubiquitous precursor farnesyl diphosphate into the first specific precursor of artemisinin, namely amorpha-4,11-diene. Here we describe the isolation of a cDNA clone encoding amorpha-4,11-diene synthase. The deduced amino acid sequence exhibits the highest identity (50%) with a putative sesquiterpene cyclase of A. annua. When expressed in Escherichia coli, the recombinant enzyme catalyzes the formation of amorpha-4,11-diene from farnesyl diphosphate. Introduction of the gene into tobacco (Nicotiana tabacum L.) resulted in the expression of an active enzyme and the accumulation of amorpha-4,11-diene ranging from 0.2 to 1.7 ng per g fresh wt.
REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:826620 HCAPLUS
DOCUMENT NUMBER: 134:189822
TITLE: Amorpha-4,11-diene Synthase of Artemisia annua: cDNA Isolation and Bacterial Expression of a Terpene Synthase Involved in Artemisinin Biosynthesis
AUTHOR(S): Chang, Yung-Jin; Song, Seung-Hwan; Park, Si-Hyung; Kim, Soo-Un
CORPORATE SOURCE: School of Agricultural Biotechnology and the Research Center for New Biomaterials in Agriculture, Seoul National University, Suwon, 441-744, S. Korea
SOURCE: Archives of Biochemistry and Biophysics (2000), 383(2), 178-184
CODEN: ABBIA4; ISSN: 0003-9861
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Artemisia annua, an indigenous plant to Korea, contains an antimalarial sesquiterpene, artemisinin. The first committed step of artemisinin biosynthesis is the cyclization of farnesyl diphosphate by a sesquiterpene synthase to produce an amorphane-type ring system. The aims of this research were to molecularly clone and express amorpha-4,11-diene synthase for metabolic engineering. PCR amplification of genomic DNA with a pair of primers, designed from the conserved regions of sesquiterpene synthases of several plants, produced a 184-bp DNA fragment. This fragment was used in Northern blot anal. as a probe, showing approx. 2.2 kb of a single band. Its sequence information was used to produce 2106 bp of a full-length cDNA sequence including 1641 bp of open reading frame for 546 amino acids (kcs12) through a rapid amplification of cDNA ends (RACE). The deduced amino acid sequence displayed 36% identity with 5-epi-aristolochene synthase of Nicotiana tabacum. A sol. fraction of Escherichia coli harboring kcs12 catalyzed the cyclization of farnesyl diphosphate to produce a sesquiterpene, which was identified through GC-MS anal. as amorpha-4,11-diene. (c) 2000 Academic Press.
REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:653514 HCAPLUS
DOCUMENT NUMBER: 134:26821
TITLE: Molecular Cloning, Expression, and Characterization of Amorpha-4,11-diene Synthase, a Key Enzyme of Artemisinin Biosynthesis in Artemisia annua L.
AUTHOR(S): Mercke, Per; Bengtsson, Marie; Bouwmeester, Harro J.; Posthumus, Maarten A.; Brodelius, Peter E.
CORPORATE SOURCE: Department of Plant Biochemistry, Lund University,

Lund, 22100, Swed.
SOURCE: Archives of Biochemistry and Biophysics (2000), 381(2), 173-180
CODEN: ABBIA4; ISSN: 0003-9861
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In plants, sesquiterpenes of different structural types are biosynthesized from the isoprenoid intermediate farnesyl diphosphate. The initial reaction of the biosynthesis is catalyzed by sesquiterpene cyclases (synthases). In Artemisia annua L. (annual wormwood), a no. of such sesquiterpene cyclases are active. We have isolated a cDNA clone encoding one of these, amorpha-4,11-diene synthase, a putative key enzyme of artemisinin biosynthesis. This clone contains a 1641-bp open reading frame coding for 546 amino acids (63.9 kDa), a 12-bp 5'-untranslated end, and a 427-bp 3'-untranslated sequence. The deduced amino acid sequence is 32 to 51% identical with the sequence of other known sesquiterpene cyclases from angiosperms. When expressed in Escherichia coli, the recombinant enzyme catalyzed the formation of both olefinic (97.5%) and oxygenated (2.5%) sesquiterpenes from farnesyl diphosphate. GC-MS anal. identified the olefins as (E)-.beta.-farnesene (0.8%), amorpha-4,11-diene (91.2%), amorpha-4,7(11)-diene (3.7%), .gamma.-humulene (1.0%), .beta.-sesquiphellandrene (0.5%), and an unknown olefin (0.2%) and the oxygenated sesquiterpenes as amorpha-4-en-11-ol (0.2%) (tentatively), amorpha-4-en-7-ol (2.1%), and .alpha.-bisabolol (0.3%) (tentatively). Using geranyl diphosphate as substrate, amorpha-4,11-diene synthase did not produce any monoterpenes. The recombinant enzyme has a broad pH optimum between 7.5 and 9.0 and the Km values for farnesyl diphosphate, Mg2+, and Mn2+ are 0.9, 70, and 13 .mu.M, resp., at pH 7.5. A putative reaction mechanism for amorpha-4,11-diene synthase is suggested. (c) 2000 Academic Press.
REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:11402 HCAPLUS
DOCUMENT NUMBER: 132:178055
TITLE: Amorpha-4,11-diene synthase catalyzes the first probable step in artemisinin biosynthesis
AUTHOR(S): Bouwmeester, Harro J.; Wallaart, T. Eelco; Janssen, Michiel H. A.; Van Loo, Bert; Jansen, Ben J. M.; Posthumus, Maarten A.; Schmidt, Claus O.; De Kraker, Jan-Willem; Konig, Wilfried A.; Franssen, Maurice C. R.
CORPORATE SOURCE: Research Institute for Agrobiology and Soil Fertility (AB-DLO), Wageningen, 6700 AA, Neth.
SOURCE: Phytochemistry (1999), 52(5), 843-854
CODEN: PYTCAS; ISSN: 0031-9422
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The endoperoxide sesquiterpene lactone artemisinin (I) and its derivs. are a promising new group of drugs against malaria. Artemisinin is a constituent of the annual herb Artemisia annua L. So far only the later steps in artemisinin biosynthesis - from artemisinic acid (II)- have been elucidated and the expected olefinic sesquiterpene intermediate has never been demonstrated. In pentane exts. of A. annua leaves we detected a sesquiterpene with the mass spectrum of amorpha-4,11-diene (III). Synthesis of III from artemisinic acid confirmed the identity. In addn. we identified several sesquiterpene synthases of which one of the major activities catalyzed the formation of III. This enzyme was partially purified and shows the typical characteristics of sesquiterpene synthases, such as a broad pH optimum around 6.5-7.0, a mol. mass of 56 kDa, and a Km of 0.6 .mu.M. The structure and configuration of III, its low content in A. annua and the high activity of amorpha-4,11-diene synthase all support

that III is the likely olefinic sesquiterpene intermediate in the biosynthesis of artemisinin.

REFERENCE COUNT: 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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(FILE 'HOME' ENTERED AT 13:49:35 ON 19 APR 2004)

FILE 'STNGUIDE' ENTERED AT 13:49:43 ON 19 APR 2004

FILE 'HOME' ENTERED AT 13:49:47 ON 19 APR 2004

FILE 'MEDLINE, HCAPLUS' ENTERED AT 13:50:08 ON 19 APR 2004

L1 14 S AMORPHA-4, 11-DIENE SYNTHASE
L2 9 DUP REM L1 (5 DUPLICATES REMOVED)
L3 4 S AMORPHADIENE SYNTHASE
L4 4 DUP REM L3 (0 DUPLICATES REMOVED)

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	40.53	41.01

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-8.32	-8.32

STN INTERNATIONAL LOGOFF AT 13:52:58 ON 19 APR 2004